INSTRUCTIONS

Pierce™ High Capacity Streptavidin Chromatography Cartridge

87739 87740

2165.1

Number	Description
87739	**Pierce High Capacity Streptavidin Chromatography Cartridge,** 2 × 1mL
87740	**Pierce High Capacity Streptavidin Chromatography Cartridge,** 1 × 5mL
	Support: 6% highly crosslinked fast-flow agarose supplied in 0.02% sodium azide
	Binding Capacity: ≥ 10mg biotinylated BSA/ml resin; ≥ 100µg biotin para-nitrophenyl ester/ml resin
	Storage: Upon receipt store at 4-8°C. Product shipped at ambient temperature. Do not freeze.

Introduction

The Thermo Scientific™ Pierce™ High Capacity Streptavidin Chromatography Cartridges are convenient, ready-to-use devices for separating biotinylated molecules from non-biotinylated molecules and for purifying antigens using biotinylated antibodies. This resin has 2-3 times higher binding capacity than the standard streptavidin agarose resin and is ideal when a high amount of recovery is required. The cartridges are effective for assay development and immunoprecipitation[1-3] and for physically separating DNA strands produced in a polymerase chain reaction by incorporating biotin in one of the amplification polymers.[4]

These cartridges enable fast, easy and reproducible chromatographic separations and are compatible with the major automated liquid-chromatography systems or manual syringe processing (see Table 1 for cartridge general properties). The cartridges attach directly to ÄKTA™ or FPLC Systems without additional connectors. The included accessory pack readily adapts cartridges for use with Luer-Lok™ Syringe Fittings or 1/16”tubing. These cartridges enable fast, easy and reproducible chromatographic separations and can be regenerated multiple times for affinity purification.

Table 1. Properties of the Thermo Scientific Pierce High Capacity Streptavidin Chromatography Cartridges.

Particle Size	45-165µm
Cartridge Dimensions	0.7 × 2.7cm (1mL column)
	1.3 × 3.8cm (5mL column)
Void Volume	0.32mL (1mL column)
	1.5mL (5mL column)
Recommended Flow Rate	0.1-1mL/min (1mL column); 0.5-2mL/min (5mL column)
Maximum Recommended Flow Rate*	4mL/min (1mL column); 5mL/min (5mL column)
pH Limits	2-11 (short-term); 4-9 (long-term)
Maximum Operating Pressure	0.3 MPa, 43.5 psi or 3 bar
Cartridge Material	Polypropylene
Frit	Polyethylene, 10µm
Accessory Pack	Luer-Lok Adapter to 10-32 male
	Finger-tight 10-32 connector fitting for 1/16” OD tubing Column plug for 10-32 coned port Cap 1/16 male

*At room temperature using water

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Important Product Information

- The example protocols included in these instructions describe applications using liquid chromatography (LC) systems using 1/16" tubing or syringes. For best results, process all buffers through a 0.45µm filter before use in LC systems. Additional connectors and fittings are required to attach these cartridges to the Bio-Rad BioLogic™ System. Specific applications and systems will require optimization.
- For optimum binding of the biotinylated molecule, apply sample at the indicated low flow rates.
- All purification procedures listed can be performed using a syringe fitted with the Luer-Lok Adapter included in the accessory pack.
- To elute biotinylated molecules from the streptavidin cartridges, use 8M guanidine•HCl, pH 1.5 (Product No. 24115). For non-denaturing elution conditions, biotinylate the protein using NHS-Iminobiotin (Product No. 21117), which binds to streptavidin at pH 9.5 and dissociates at pH 4. Alternatively, use a thiol-cleavable biotinylation reagent such as NHS-SS-biotin (Product No. 21331) or NHS-SS-PEG_4-biotin (Product No. 21442).
- Guanidine•HCl is a harsh denaturant and may irreversibly damage the protein of interest and result in leaching of streptavidin subunits. The cartridge cannot be reused after eluting with guanidine•HCl.
- The cartridge was not designed to be refilled.

Procedure for Purifying Biotinylated Molecules

A. Additional Materials Required

- Binding Buffer: Phosphate-buffered saline (e.g., 0.1M phosphate, 0.15M sodium chloride; pH 7.2; Product No. 28372)
- Elution Buffer: 8M guanidine•HCl, pH 1.5 (Product No. 24115)

B. Procedure

Note: For syringe application, 30 drops per minute is equivalent to a flow rate of 1mL per minute.

1. Use approximately 10mg of biotinylated protein/ml of streptavidin resin. Adjust the sample to the ionic strength and pH of the Binding Buffer by dilution or buffer exchange before applying to the cartridge. If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) before use.
2. Equilibrate the cartridge and all buffers to working temperature. Perform purifications at room temperature or 4°C. Ensure that all solutions are degassed.
3. Prepare the LC system by filling tubing with buffer. Remove top plug from cartridge and carefully snap off the end-tab (do not twist). To avoid introducing air into the system, let a few drops of buffer flow from tubing into the cartridge then connect cartridge top to the tubing; allow a few drops to emerge from the cartridge before connecting to the LC inlet port.
4. Equilibrate the cartridge with 10 column volumes of Binding Buffer at a flow rate of 0.1-1mL/minute for the 1mL cartridge or 0.5-2mL/minute for the 5mL cartridge.
5. Apply biotinylated sample to the cartridge. Use a 0.1-0.2mL/minute flow rate for a 1mL cartridge or 0.5-1mL/minute for a 5mL cartridge. Apply any volume that does not exceed cartridge capacity. Collect fractions.
6. Wash with at least 10 column volumes of Binding Buffer or until the absorbance of the flow-through approaches baseline. Use 0.1-1mL/minute or 0.5-2mL/minute flow rate for washing 1mL or 5mL columns, respectively.
7. Elute the bound biotinylated sample with approximately 10-20 column volumes of Elution Buffer and collect fractions. Immediately dialyze or buffer exchange the fractions of interest.
8. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by performing a protein assay (e.g., Thermo Scientific BCA Protein Assay, Product No. 23227).

Procedure for Affinity Purification of Antigens

A. Additional Materials Required

- Biotinylated antibody: Prepare biotinylated antibody at 0.2-10mg/mL in Binding Buffer and use approximately 10 mg of biotinylated antibody/ml of streptavidin resin
- Antigen-containing sample
- Binding Buffer: Phosphate-buffered saline (e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372)
- Elution Buffer: IgG Elution Buffer (Product No. 21004), Gentle Ag/Ab Elution Buffer (Product No. 21027) or 0.1M glycine•HCl, pH 2.8

B. Procedure

Note: For syringe application, 30 drops per minute is equivalent to a flow rate of 1mL per minute.

1. Equilibrate the cartridge and all buffers to working temperature. Perform purifications at room temperature or 4°C. Ensure that all solutions are degassed.
2. Prepare the LC system by filling tubing with buffer. Remove top plug from cartridge and carefully snap off the end-tab (do not twist). To avoid introducing air into the system, let a few drops of buffer flow from the tubing into the cartridge and then connect cartridge top to the tubing; allow a few drops to emerge from the cartridge before connecting to the LC inlet port.
3. Equilibrate the cartridge with 10 column volumes of Binding Buffer at a flow rate of 0.1-1mL/minute for the 1mL cartridge or 0.5-2mL/minute for the 5mL cartridge.
4. Apply biotinylated antibody solution to the cartridge. Use a 0.1mL/minute flow rate for a 1mL cartridge or 0.5mL/minute for a 5mL cartridge. Alternatively or for syringe application allow one column volume of solution to enter the cartridge. Pause the flow and incubate for 10-15 minutes. If the entire solution volume cannot be added at once, repeat with more antibody solution and incubate. Apply any volume that does not exceed cartridge capacity.
5. Wash with at least 10 column volumes of Binding Buffer or until the flow-through absorbance approaches baseline. Use a flow rate of 0.1-1mL/minute for the 1mL cartridge or 0.5-2mL/minute for the 5mL cartridge.
6. Apply antigen sample to the cartridge at 0.1mL/minute flow rate for a 1mL cartridge or 0.5mL/minute for a 5mL cartridge. Alternatively or for syringe application allow the sample to enter the cartridge, pause the flow and incubate at room temperature for 30 minutes or overnight at 4ºC.
7. Wash with at least 10 column volumes of Binding Buffer or until the flow-through absorbance approaches baseline. Use a flow rate of 0.1-1mL/minute for the 1mL cartridge or 0.5-2mL/minute for the 5mL cartridge.

   Note: If using Gentle Ag/Ab Elution Buffer, wash cartridge with three column volumes of Tris-buffered saline before antigen elution. The Gentle Elution Buffer is not compatible with phosphate-based buffers.
8. Elute the bound antigen with approximately 10-20 column volumes of Elution Buffer and collect fractions.
9. If using IgG Elution Buffer or 0.1M glycine•HCl, pH 2.8, immediately adjust the pH to neutral by adding 100µL of 1M Tris; pH 7.5-9.0 per 1mL of eluate collected or by dialysis or buffer exchange.
10. Monitor protein elution by measuring the absorbance of the fractions at 280nm or by using the BCA Protein Assay. The eluted protein can be directly analyzed by SDS-PAGE.
11. Re-equilibrate the immobilized antibody cartridge with at least 10 column volumes of Binding Buffer until the flow-through absorbance returns to baseline. The ability to reuse the cartridge is dependent upon each specific immobilized antibody and the sample. For best results reuse cartridge with identical samples to minimize contamination. Store cartridge at 4ºC in 0.02% sodium azide.

Procedure for Purifying Iminobiotinylated Molecules

A. Additional Materials Required

- NHS-Iminobiotin-labeled sample adjusted to the ionic strength and pH of the Binding Buffer
- Binding Buffer: 50mM ammonium carbonate, 0.5M NaCl, pH 10.0
- Elution Buffer: 50mM ammonium acetate, 0.5M NaCl, pH 4.0

B. Procedure

Note: For syringe application, 30 drops per minute is equivalent to a flow rate of 1mL per minute.

1. Equilibrate the cartridge and all buffers to working temperature. Perform purifications at room temperature or 4°C. Ensure that all solutions are degassed.
2. Prepare the LC system by filling tubing with buffer. Remove top plug from cartridge and carefully snap off the end-tab (do not twist). To avoid introducing air into the system, let a few drops of buffer flow from tubing into cartridge top then connect cartridge top to the tubing; allow a few drops to emerge from the cartridge before connecting to the LC inlet port.
3. Equilibrate the cartridge with 10 column volumes of Binding Buffer at a flow rate of 0.1-1mL/minute for the 1mL cartridge or 0.5-2mL/minute for the 5mL cartridge.
4. Apply iminobiotinylated sample to the cartridge at 0.1-0.2mL/minute flow rate for a 1mL cartridge or 0.5-1mL/minute for a 5mL cartridge. Apply any volume that does not exceed cartridge capacity.
5. Wash with at least 10 column volumes of Binding Buffer or until the absorbance of the flow-through approaches baseline. Use 0.1-1mL/minute or 0.5-2mL/minute flow rate for washing 1mL or 5mL columns, respectively.
6. Elute with approximately 10-20 column volumes of Elution Buffer and collect fractions.
7. Immediately adjust the pH of the eluate to neutral by buffer exchange.
8. Monitor protein elution by measuring the absorbance of the fractions at 280nm or using the BCA Protein Assay. The eluted protein can be directly analyzed by SDS-PAGE.
9. Typically the cartridge can be reused multiple times. For best results, reuse cartridge with identical samples to minimize contamination. Store cartridge at 4ºC in 0.02% sodium azide. For reuse, equilibrate the cartridge with at least 10 column volumes of Binding Buffer until the flow-through absorbance returns to baseline.

Related Thermo Scientific Products

87741	**Pierce High Capacity NeutrAvidin Chromatography Cartridges,** 2 × 1mL
87742	**Pierce High Capacity NeutrAvidin Chromatography Cartridges,** 2 × 5mL
21117	**NHS-Iminobiotin Trifluoracetamide**
21435	**EZ-Link™ Sulfo-NHS-LC-Biotinylation Kit**
21440	**EZ-Link NHS-PEG Solid Phase Biotinylation Kit: *pre-packed column***
20227	**Pierce Monomeric Avidin Kit**
66425	**Slide-A-Lyzer™ Dialysis Cassette, 10K MWCO, 3mL,** 10 pack
89934	**Pierce Desalting Chromatography Cartridges,** 1 ml × 5 columns
89935	**Pierce Desalting Chromatography Cartridges,** 5 ml × 5 columns
89891	**Zeba™ Spin Desalting Columns, 7K MWCO, 5mL,** 5/pkg
89893	**Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 5/pkg
89894	**Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 25/pkg

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- Tech Tip #27: Optimize elution conditions for immunoaffinity purification
- Tech Tip #29: Degas solutions for use in affinity and gel filtration columns

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General References

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